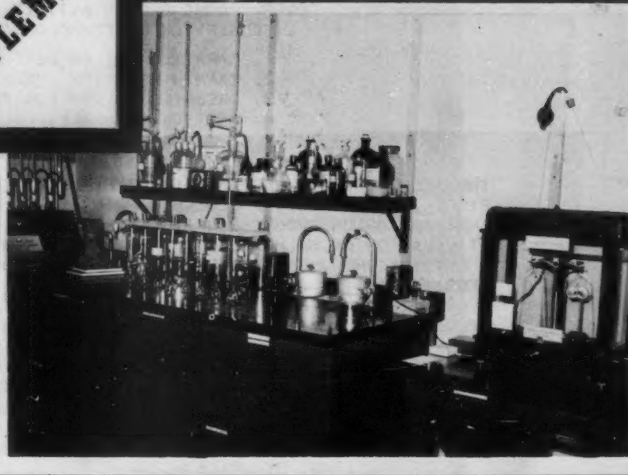
COMMERCIAL FISHERIES REVIEW

TECNOLOGY SUPPLEMENT



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United States Department of the Interior
Washington, D.C.

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DEPARTMENT OF THE INTERIOR
OSCAR L. CHAPMAN, Secretary

FISH AND WILDLIFE SERVICE
ALBERT M. DAY, Director

COMMERCIAL FISHERIES REVIEW

A REVIEW OF DEVELOPMENTS AND NEWS OF THE FISHERY INDUSTRIES
PREPARED IN THE BRANCH OF COMMERCIAL FISHERIES

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′# COMMERCIAL FISHERIES REVIEW

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THE TECHNOLOGICAL SECTION AIDS THE FISHERIES

The functions of the Technological Section (one of six[1] sections in the Branch of Commercial Fisheries, U. S. Fish and Wildlife Service) consist of research and field services for the fisheries and allied industries. The research activities include field and laboratory investigations in chemistry, pharmacology, nutrition, biochemistry, and bacteriology.

Four research laboratories are maintained by the Technological Section. These are located in Boston, Mass.; College Park, Md.; Ketchikan, Alaska; and Seattle,

FISHERY TECHNOLOGICAL LABORATORY IN COLLEGE PARK, MD., LOCATED ON THE CAMPUS OF THE UNIVERSITY OF MARYLAND. (THE LABORATORY SERVING THE NEW ENGLAND AREA IS LOCATED IN THE APPRAISERS STORES BUILDING, IN BOSTON, MASS.

1/THE OTHER SECTIONS OF THE BRANCH ARE ECONOMICS AND COOPERATIVE MARKETING; EDUCATIONAL AND MARKET DEVELOPMENT; EXPLORATORY FISHING AND GEAR DEVELOPMENT; FISHERY MARKET NEWS; AND STATISTICAL.

Washington. A fifth laboratory in Mayaguez, Puerto Rico, is no longer operating because of lack of funds.

The Section is particularly concerned with research and development problems peculiar to the fishery industries; i.e., the utilization of fishery products.

FISHERY TECHNOLOGICAL LABORATORY IN SEATTLE, WASH., WHERE BOTH TECHNOLOGICAL AND BIOLOGICAL FISHERIES RESEARCH ARE CONDUCTED.

In general, the specific activities of each of the laboratories are the same. These include studies on preservation, freezing, canning, sanitation, nutritive value, bacteriology, pharmacology, chemistry, and utilization of fishery byproducts. Mobile or trailer laboratories are maintained to enable chemical and bacteriological studies of the fisheries in the field in out-of-the-way places.

Funds for technological work are allotted on a nation-wide basis and must be used to carry out studies of greatest importance to the industry as a whole. Besides these research and development studies, consulting services of the Section are at all times available to the entire industry on nearly all phases of commercial fisheries.

At the beginning of each fiscal year (July 1), the chiefs of each of the technological laboratories meet in Washington to discuss the progress on the preceding year's program and to develop a new program for the next fiscal year. Members of the fishing and allied industries are invited to attend the meeting and to offer their comments, criticisms, and suggestions. These meetings with members of industry have aided the Branch considerably in establishing the commercial fisheries technological research program on as sound a basis as funds and facilities permit. The last meeting was held on June 28, 1950, when the following program was developed for the fiscal year 1950-51, beginning July 1, 1950.

FISHERY TECHNOLOGICAL RESEARCH PROGRAM, 1950-51

Nutrition

1. *Effect of low and high temperatures on chemical properties of proteins.--An investigation will be made into the way in which water is bound to protein. When fish is frozen the first change is separation of the water from the protein. Upon thawing there is the question of whether the water returns to its original relationship to protein. The study will include some preliminary work on isolation and identification of fish proteins and possibly a study of some of their properties and reactions, such as, isoelectric point, heat denaturation, etc. These investigations might lead to important advances toward an understanding of the nature and control of drip, possibly information on the toughening of fish in cold storage, etc., and would also be of importance to drying of fish, such as fish meal, and dehydration of fish. (Seattle)

2. Utilization of salmon cannery waste for hatchery food.--Work of the preceding year was concerned with:

(a) Collaborative work with other State and Federal agencies in the analysis of mixed diets and special diet components for chemical proximate analyses and vitamin content;

FISHERY PRODUCTS LABORATORY IN KETCHIKAN, ALASKA. OPERATED JOINTLY BY THE FISH AND WILDLIFE SERVICE AND THE FISHERIES EXPERIMENTAL COMMISSION OF ALASKA.

* NEW PROJECT.

(b) Collection of raw material such as hake and other fish for hatchery feeding tests; and

(c) Preservation and processing of raw material, particularly tests on salmon eggs at relatively low temperatures using various chemical preservatives and antioxidants.

In view of the promising results in feeding tests with salmon eggs, the studies will be continued with special emphasis on completing the work on the preservation of salmon eggs. Suitable methods of preserving salmon eggs for hatchery food would allow the tapping of a considerable portion of the Alaska resources, now commonly wasted, and which totals in excess of 8,500,000 pounds annually. (Seattle)

3. Clam processing methods and clam toxicity survey.--Tests indicate in part:

(a) That toxin is present in butter clams of Alaska from certain areas during every month;

(b) The siphons are several times greater in toxicity than are the bodies of the clams;

(c) Rate of toxin loss in highly toxic clams transplanted into a relatively non-toxic beach is very low; and

(d) Suitable commercial packs of canned minced clams are feasible when processed by recommended methods of the laboratory.

The remaining experimental work will be completed during the next fiscal year. (Ketchikan)

Refrigeration

1. Freezing fish at sea, defrosting, filleting, and refreezing the fillets.--Laboratory tests indicate the superiority in regard to appearance, taste, and texture of frozen fillets prepared from fish frozen in the round immediately after catching as compared to frozen fillets prepared from fish which were first gutted and iced for a 10-day period. Laboratory tests have now proceeded to a point where commercial scale tests are warranted. Additional laboratory studies will be directed toward obtaining information on:

(a) Effect of prolonged storage of frozen round fish prior to defrosting and filleting;

(b) Ratio of brine to fish necessary for optimum freezing;

(c) Absorption of brine by fish;

(d) Effect of rate of freezing or temperature of brine on quality of fish;

(e) Viscera yield from round fish; and

(f) Thawing methods. (Boston)

2. Study of fresh and frozen oysters.--This project is being continued and was initiated with a view toward developing and improving methods of freezing, packaging, storing, and testing oysters. These studies include, specifically:

(a) pH determinations of fresh and frozen shucked oysters;

(b) Prevention of discoloration in frozen oysters; and

(c) Packaging of frozen oysters. (College Park)

3. Studies of methods of handling frozen salmon to be used for canning.--Processing difficulties have developed and some commercial canned packs prepared from frozen salmon were of inferior quality. The practice of preparing packs from frozen fish is being adopted in certain instances by various processors where the fish is caught and frozen in remote areas and canned at existing canneries elsewhere. Experimental studies on frozen red (sockeye) salmon indicated that the flavor and texture of the canned red salmon prepared from the frozen fish was significantly adversely altered in comparison with the red salmon canned from fresh fish of the same lot. Additional studies will be made on the effect of storage temperature and freezing variables on the quality of the final canned product. (Ketchikan)

4. Freezing pink salmon.--Freezing tests to date indicate the improvement in keeping quality of frozen pink salmon fillets due to ascorbic acid treatment, superior packaging methods, and storage of the fillets at -20° F. The present series are to be completed. A commercial scale test is warranted and will be carried out as the interest and cooperation of the industry indicate. (Ketchikan)

5. *Freezing and storing Alaska shrimp and dungeness crabs.--Development of new and improved methods of freezing and storing Alaska shrimp and crabs is important in the development of the local industries. Studies on shrimp are to be made on a limited basis on effect of precooking variables and packaging conditions on the quality of the stored frozen shrimp. Studies on dungeness crabs will consider packaging methods with new type containers. (Ketchikan)

6. *Palatability and cold storage life of various species of Pacific Coast rockfishes.--A study will be directed to determine whether certain species of Pacific Coast rockfishes are of sufficiently superior quality to the other species to warrant separate marketing. (Seattle)

7. *Preparation of a manual on the refrigeration of fish.--The preparation of a complete manual on the refrigeration of fish has been authorized.

Processing and Preservation

1. Canning of "little tuna."--Packs to date indicate that it is possible to prepare a canned product of commercial quality. The present experimental tests will be carried out to completion. (College Park)

2. *Evaluation of the antibiotic "subtilin" as a preservative for fishery products.--Tests by the Department of Agriculture showed promising results in the use of "subtilin" in the preservation of canned fruits and vegetables. Subtilin is an antibiotic produced by some strains of the bacterium *Bacillus subtilis* under proper culturing conditions. Its experimental use as a preservative in certain canned food products allows shorter processes resulting in a quality product of

*NEW PROJECT.

better appearance and taste than those prepared by the normal processing methods. Attempts will be made to apply subtilin to the preservation of certain fishery products. (Boston, College Park, and Seattle)

Sanitation and Bacteriology

1. A bacteriological survey of the preparation of crab meat.--Limited studies begun during the last year are to be supplemented with further work to establish recommended practices for the handling of highly perishable crab meat, making use of such data on the subject as have already been collected by laboratory personnel. (College Park)

2. *Growth of pink yeast (isolated from oysters) at below freezing temperatures.--A "pink" yeast was isolated from an active culture found on oysters stored at 0° F. Further studies are to be carried out on the cultural characteristics and the physiology of the organism with a view toward its control in the oyster industry. (College Park)

Analysis and Composition

1. *Chemical composition of fish, (A) Menhaden.--Complete analyses are to be made of certain fish, beginning with menhaden, for chemical proximate analyses, proteins and protein degradation products, vitamins, and biologically active compounds with a view towards determining the over-all potential commercial value of the fish. (College Park)

2. Cooperative work with the Association of Official Agricultural Chemists on the determination of oil in fish meal.--Further tests on the improvement of methods for the determination of oil in fish meal are to be conducted in the light of knowledge gained on earlier tests and to pursue newer ideas on the subject. The present methods are inadequate and give results as much as 20 percent of true value. Accurate testing methods are necessary to avoid confusion between buyer and seller of fish meal. (Seattle)

Byproducts

1. *Vitamin content, particularly animal protein factor and vitamin B_{12} of fishery byproducts.--Analysis and feeding tests are to be conducted on fishery byproducts (fish meal and solubles) to determine the potential value of the products in animal and poultry feeding. (College Park and .eattle)

2. Utilization of salmon cannery waste for animal food--Cooperative tests with the Petersburg, Alaska, experimental fur farm.--Work will be continued on collecting analyzing, and processing salmon waste in connection with cooperative studies with the U.S. D.A., Petersburg, Alaska fur farm, on use of salmon waste as feed for fur farm animals. (Ketchikan)

3. *Preparation of a manual on the fish meal and oil industry.--The preparation of a complete manual on the fish meal and oil industry has been authorized.

* * * * *

In addition to the above projects, several carried over from the preceding year will be written up and reports completed during the first and second quarter of this fiscal year. These include:

* NEW PROJECT.

1. Determination of food value of fishery products as prepared for serving.

2. Correlation of biological and spectrophotometric methods for the determination of vitamin A potencies.

3. Effect of fluctuating temperatures on quality of frozen fish in storage and in transit.

4. Frozen storage of certain Pacific Coast fish, second report.

INFORMATION ON PROGRESS OF TECHNOLOGICAL PROJECTS

Current information regarding the progress on the various projects is presented in Commercial Fisheries Review (CFR) in the section "Research in Service Laboratories."

More detailed information on the projects may be obtained by the fishery and allied industries by writing directly to the Branch of Commercial Fisheries, Fish and Wildlife Service, Washington 25, D. C.; to the laboratories;[1] or by consulting with members of the Technological Section. Phase or final reports on projects are usually published in Commercial Fisheries Review, as fishery leaflets (FL) or scientific reports, or in non-governmental scientific journals. Abstracts of these and other current information pertaining to commercial fisheries are available in Commercial Fisheries Abstracts (CFA).

CFR, CFA, FL, and most scientific reports are available free to members of the fishery and allied industries on request. Some of the special scientific reports are sold by the Superintendent of Documents, Government Printing Office, Washington 25, D. C.

1/SEE INSIDE BACK COVER OF THIS ISSUE FOR ADDRESSES OF LABORATORIES.

PACKAGING FROZEN FISHERY PRODUCTS

The requirements for satisfactory containers for frozen fishery products are no less rigid than for other frozen products. The choice of the package is very important in protecting and merchandising the product. The problem of vital importance is that of preventing loss of moisture from the product. The use of packaging materials having low water-vapor transmission rates must be emphasized. A package made of suitable water-vaporproof materials which will satisfactorily withstand low storage temperatures without becoming brittle or otherwise unsatisfactory and which is tightly sealed, will provide adequate protection for a long period of frozen storage.

--Fishery Leaflet 324

UTILIZATION OF SALMON EGGS FOR PRODUCTION OF CHOLESTEROL, LIPIDE, AND PROTEIN

By G. Ivor Jones*, Edward J. Carrigan*, and John A. Dassow**

ABSTRACT

QUANTITATIVE DETERMINATION OF CHOLESTEROL, FAT, PHOSPHOLIPIDE, AND PROTEIN WERE CARRIED OUT ON THE ROE (EGGS) OF FIVE SPECIES OF SALMON. THE CHOLESTEROL CONTENT WAS FOUND TO VARY FROM 0.29 TO 0.40 PERCENT, ON THE RAW-EGG BASIS. WHEN CALCULATED ON THE LIPIDE FRACTION (ETHER-SOLUBLE FAT = 11.1 TO 13.9 PERCENT OF THE WHOLE EGG), THE CHOLESTEROL VARIED FROM 2.21 TO 3.53 PERCENT.

PRELIMINARY ANIMAL-FEEDING STUDIES SHOWED THAT THE DEFATTED SALMON EGG PROTEIN COMPARED FAVORABLY WITH CASEIN IN NUTRITIONAL QUALITY. PROTEIN CONTENT OF THE ROE VARIED FROM 22.5 TO 28.8 PERCENT AND THE ASH CONTENT FROM 1.3 TO 2.7 PERCENT.

PHOSPHOLIPIDE, BASED ON THE PHOSPHOROUS CONTENT OF THE LIPIDE FRACTION, RANGED FROM 10.4 TO 12.4 PERCENT OF THE EGG (MOISTURE-FREE BASIS) OR 25.8 TO 39.2 PERCENT OF THE ETHER-SOLUBLE FAT.

UTILIZATION OF SALMON EGGS FOR THE EXTRACTION OF THE RELATIVELY SMALL AMOUNT OF CHOLESTEROL PRESENT IS UNWARRANTED AT THIS TIME. HOWEVER, COMMERCIAL EXPLOITATION OF THE LIPIDE AND PHOSPHOLIPIDE FRACTIONS AND PERHAPS THE PROTEIN OF SALMON ROE APPEARS TO BE PRACTICAL.

INTRODUCTION

Attention was directed to an investigation of salmon eggs as a possible commercial source of cholesterol, lipide, and protein by the findings of a preliminary survey by Jones and Carrigan (1947) carried out during the initial stage of the research program on utilization of Alaskan salmon-cannery waste. The period of study was necessarily limited to the six months' contract of the Industrial Research and Development Division of the Office of Technical Services with the Alaska Fisheries Experimental Commission, under which the investigation was possible. Accordingly, the experimental work was arranged so that the information required to evaluate the possibilities of further development could be collected in the allotted time. From analytical data reported in the literature, the use of salmon eggs as a source of cholesterol appeared promising. It was hoped that commercial development might be practical if experimental tests showed the salmon eggs under study to be as high in cholesterol content as had previously been reported.

It was believed very likely that in addition to cholesterol extraction, processes could be developed which would also permit recovery of a high quality protein meal from salmon eggs as well as a fat or lipide fraction which might have a number of important industrial applications.

The presence of cholesterol in the roe of fish has been reported on by several investigators. Koenig and Grossfeld (1913) reported that the fat from fish roe contains from 4 to 14 percent cholesterol. Anno (1940) found that the unsaponifiable matter present in the lipides of salmon eggs was essentially cholesterol. In addition to cholesterol, the lipide fraction of fish roe has a

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high content of lecithin. Koenig and Grossfeld (1913) in considering fish roe as food for man found the egg fat to contain as much as 49 percent lecithin. Halpern (1945) reported that the roe from sockeye salmon (Oncorphynchus nerka) yielded 12.5 percent oil and 6.2 percent phospholipide.

The separation of lecithin from the extracted egg fat would appear to be easily accomplished by a process developed by the German oil industry and described by Goss (1947). In this process, the lecithin is removed from the crude fat or oil by washing it with 2 to 5 percent by volume of hot water and removing the resulting sludge in a centrifuge. Two successive washings are required to insure maximum recovery of lecithin. The lecithin is recovered from the sludge by removal of water at 60° C. with the aid of vacuum, followed by a bleaching of the residue with hydrogen peroxide. Residual oil remaining in the lecithin is then recovered by repeated extractions with acetone.

Recovery of a semipurified protein of high nutritional quality may have considerable importance in the economic utilization of salmon eggs. The protein content of salmon eggs varies from about 22 percent in king or chinook salmon to about 28 percent in the chum.[1]

The quantities of salmon eggs available in Alaska for processing are enormous. Since the eggs constitute about 8 to 10 percent of the entire salmon-cannery waste, which amounts to more than 100,000,000 pounds annually, it can be readily calculated that about 9,000,000 pounds or 4,500 tons of salmon eggs are at present discarded each year.

Collection of the raw salmon eggs in Alaska should not interpose any very difficult problems. The salmon are dressed prior to canning in a machine known as the "Iron Chink" which in a single cycle cuts off the head, fins, tail and removes the viscera. The eggs as a part of the viscera, are swept out of the body cavity in the middle cycle of the rotating wheel of the "Iron Chink." Separation of the eggs from the rest of the abdominal contents would necessitate hand sorting to only a moderate degree. The large and, in most cases, intact skeins of salmon eggs could be readily separated from the other waste parts while they are traveling along a belt, chute, or trough.

Problems of handling and storing salmon eggs for subsequent processing are expected to be somewhat easier to overcome than those of other fractions of salmon-cannery waste, because the eggs are individually encased in a tough semipermeable membrane and the entire egg mass is held together in a skein structure which offers ease of handling and some protection from contamination. If it were found necessary to hold or store the salmon eggs for a considerable period of time before processing, this no doubt could be accomplished by salting, freezing, or by addition of a chemical preservative. Salmon eggs appear to offer a unique material for chemical processing due to their special constituents and because of the size of the roe in salmon waste and the enormous quantity that is available in Alaska.

EXPERIMENTAL PROCEDURES

Before accurate assessment of the possibility of recovering cholesterol from salmon eggs could be made, it was necessary to determine the quantity of cholesterol present in this portion of the cannery waste as it occurs in Alaska. Since a complete survey of the variation in cholesterol content due to size of fish, maturity, and location of capture, would require an expenditure of a large

1/ UNPUBLISHED DATA OF THE AUTHORS.

amount of time and money, it was decided to limit the preliminary analysis to a sample of eggs from 25 to 100 fish of each species. It was believed this sample would furnish a fairly representative approximation of the cholesterol content to be encountered.

The egg samples for the analyses presented in the following report for all species of salmon, except king or chinook, were collected during the 1947 fishing season at two salmon canneries located at Ketchikan, Alaska. The samples of king eggs were collected from Columbia River chinook salmon at a cannery located at Astoria, Oregon. Each sample of approximately 35 pounds of raw eggs represented the roe from 25 to 100 salmon of the individual species. Samples of each species other than king were obtained directly from the "Iron Chink" butchering operation with no attempt to segregate the material according to size or maturity. The salmon were trap-caught, in most instances, and represented fish of average size which were semi-mature, as evidenced by development of the gonads. The eggs were inspected for the presence of other waste parts before being sealed in five-gallon tin containers and frozen in a sharp freezer at -20° F., within 4 hours after collection and about 24-36 hours after the salmon were caught. All samples were held at 0° F. storage until thawed and ground prior to chemical analysis.

TABLE 1 - AVERAGE CHOLESTEROL AND FAT CONTENT OF SALMON EGGS

SPECIES OF SALMON	CHOLESTEROL		FAT 1/
	IN RAW EGGS	IN FAT	
	PERCENT	PERCENT	PERCENT
PINK	0.29	2.61	11.1
RED	0.39	2.82	13.9
CHUM	0.38	3.15	11.9
CHUM, DEHYDRATED	0.86	3.06	28.0
KING	0.34	2.64	12.8
COHO	0.40	3.53	11.4

1/ TOTAL ETHER EXTRACT AFTER ACID HYDROLYSIS OF SAMPLE.

Each analysis in Table 1 was made on a representative sample drawn from the entire 35-pound lot of thoroughly mixed ground eggs. This small representative sample of about 250 to 300 grams was blended in a Waring Blendor prior to removal of a sample for the determination of cholesterol and ether-soluble fat. The value reported for king or chinook eggs was determined on a representative sample drawn from a 35-pound lot collected at Astoria, Oregon, during August 1947. Due to the large size of this species, a 35-pound sample of eggs represents only 15 to 20 fish.

CHOLESTEROL DETERMINATION

Numerous methods for the quantitative determination of cholesterol are found in the chemical literature. However, many of these methods are modifications of the Lieberman-Burchard reaction, and are designed primarily for the determination of small quantities of cholesterol present in blood. Methods commonly used for the determination of cholesterol in hen egg yolks or in other food products containing egg yolk appeared to be the most logical to use for the analysis of salmon eggs. Accordingly, the method described in Methods of Analysis of the Association of Official Agricultural Chemists, VI Edition, 1945, page 349, for determination of cholesterol in eggs and egg products was used in the initial experiments on salmon eggs. In this method the cholesterol is isolated from a saponified sample as the dibromide and subsequently determined by an iodine liberation-titration method using sodium thiosulphate. This method is considered precise and accurate, but has the disadvantage of being laborious and time consuming. In order to examine a larger number of samples, a simpler method was resorted to after a preliminary check analysis had been made using an aliquot of the same sample in the determination of cholesterol by both the A.O.A.C. VI method and the colorimetric method of Cook and Mehlenbacher (1946). The Cook and Mehlenbacher method is based on the Lieberman-Burchard color reaction for cholesterol using the unsaponifiable fraction of the ether extract. Cook and Mehlenbacher suggested the use of a lower temperature during color development

and subsequent reading and also the use of a spectrophotometer to obtain the transmittance values.

Initially the cholesterol content of dehydrated (lyophilized) chum salmon eggs was determined by the A.O.A.C. method. The amount of cholesterol calculated on the basis of the total oil fraction was found to agree within the limits of experimental error with that found for the oil from raw eggs when analyzed by the colorimetric method. Subsequent values for cholesterol in salmon eggs were determined in duplicate by the colorimetric procedure. It was not necessary to use more than two to three grams of ground salmon eggs for each analysis. With this small sample, it was possible to saponify the eggs directly by addition of 30 ml. of 95 percent ethanol and 3 ml. of 50 percent KOH followed by refluxing on a steam bath for 30 minutes. The combined ether extractions of the unsaponifiable fraction were washed with distilled water until the washings were neutral to phenolphthalein. The extract was then made up to a volume of 100 ml. with ethyl ether. Five ml. aliquots were placed in dry test tubes for color development; the ether was removed by immersion in a water bath maintained at 60° C., and 5 ml. of C. P. chloroform were added when the ether had evaporated. The color was developed at 18° C. for 25 minutes in accordance with the Cook-Mehlenbacher technique using acetic anhydride-sulfuric acid mix. The period of color development was not critical as it was found that a period from 20 to 30 minutes gave reasonable good agreement on replicates. Transmittance values were determined at 640 mmu. with a Beckman spectrophotometer using 1 cm. corex cells. Values were obtained from the transmittance-concentration curve with a range of 0.2 to 0.60 mg. cholesterol per 5 ml. chloroform. Blank determinations showed no absorption caused by impurities in the reagents used.

Transmittance values for known amounts of cholesterol subjected to color development are shown in Figure 1.

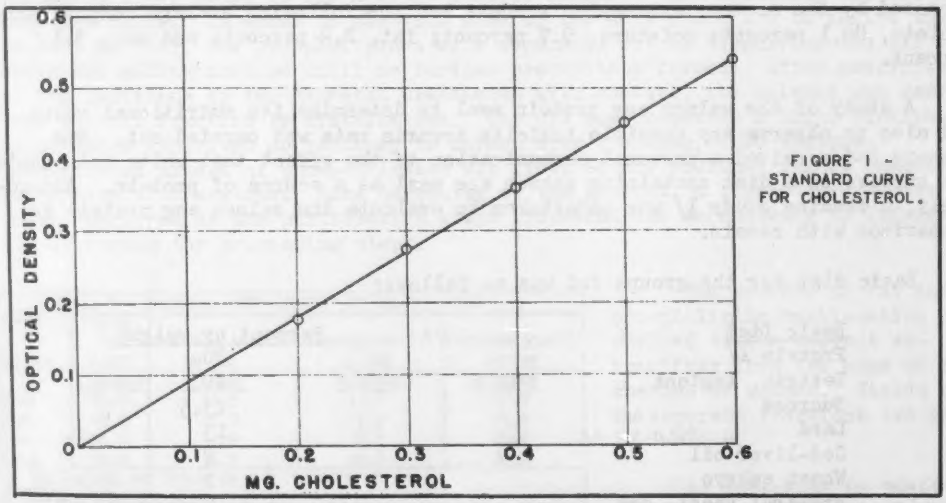

FIGURE 1 - STANDARD CURVE FOR CHOLESTEROL.

The values for cholesterol present in salmon eggs of five different species of salmon are given in Table 1.

These data indicate that the cholesterol content of salmon eggs lies near the lower part of the range of 4 to 14 percent cholesterol in fish-roe fat as reported in the literature. For comparison purposes, it is pointed out that the fat fraction of hens' eggs contains an average of 4.24 percent cholesterol as compared with 3.53 percent cholesterol in the fat of coho salmon eggs.

Anno (1) reported that the unsaponifiable matter extracted from the eggs of pink salmon was essentially cholesterol. Observations made during the present study failed to confirm this report. As indicated in Table 2, approximately one-half of the unsaponifiable matter did not respond to the reactions for cholesterol.

TABLE 2 - CHOLESTEROL CONTENT OF THE UNSAPONIFIABLE MATTER OF SALMON EGGS

SPECIES OF SALMON	UNSAPONIFIABLE RESIDUE 1/ IN FAT	CHOLESTEROL IN FAT	CHOLESTEROL IN THE UNSAPONIFIABLE RESIDUE
	PERCENT	PERCENT	PERCENT
PINK	5.44	2.16	48
RED	4.44	2.82	64
CHUM	6.46	3.15	49
KING	5.16	2.69	52
COHO	7.10	3.53	50

1/ UNSAPONIFIABLE RESIDUE DETERMINED BY A.O.A.C. VI METHODS OF ANALYSIS.

PROTEIN, FAT, AND LECITHIN RECOVERY

In order to obtain the lipide fraction and protein fraction of salmon eggs for evaluation, an extraction process was developed using acetone directly on the raw salmon eggs. In this process, the acetone removes the water content of the egg and a major portion of the fat. Final extraction of some of the remaining lipoidal material is accomplished with hot ethanol. By distillation of the acetone-water-fat solution at atmospheric pressure, the solvent is recovered and the oil or fat separates as a layer on top of the water in the still pot. The oil is then separated from the water by decantation.

The pilot plant studies carried out on the acetone extraction of salmon eggs will not be included in this report. A sufficiently large quantity of protein meal and salmon egg fat were prepared for evaluation studies. The protein meals prepared by the acetone extraction process had the following average composition: protein, 84.1 percent; moisture, 9.7 percent; fat, 2.4 percent; and ash, 3.1 percent.

A study of the salmon egg protein meal to determine its nutritional value and also to observe any possible toxicity towards rats was carried out. The authors had received a personal communication to the effect that white rats could not survive on a diet containing salmon egg meal as a source of protein. Accordingly, a feeding study 1/ was undertaken to evaluate the salmon egg protein in comparison with casein.

Basic diet for the groups fed was as follows:

Basic Diet	Percent by weight
Protein 2/	30
Dextrin, tapioca	20
Sucrose	25.5
Lard	15
Cod-liver oil	2
Wheat embryo	2
Brewers' yeast, dry	2
Liver extract, Lilly	0.5
Mineral mixture, USP XIII, No. 2	3

1/ CARRIED OUT AT THE SERVICE'S COLLEGE PARK FISHERY TECHNOLOGICAL LABORATORY.

2/ FOR THE CONTROL GROUP, 25-PERCENT TECHNICAL CASEIN AND 5-PERCENT DEXTRIN WERE SUBSTITUTED FOR THE 30-PERCENT SALMON EGG PROTEIN.

White rats were allotted to the two groups at random and kept in individual cages. Food and water were allowed ad libitum. Data, including rat weight and food consumption, were recorded weekly. Gain in body weight to food consumption was calculated and is presented in Table 3.

TABLE 3 - EVALUATION OF SALMON EGG PROTEIN MEAL FOR GROWTH OF RATS

DIET DESIGNATION	SEX	INITIAL WEIGHT	LENGTH OF EXPERIMENT	GAIN IN LIVEWEIGHT	FOOD CONSUMED	RATIO OF GRAMS OF FOOD TO GRAMS OF GAIN IN WEIGHT OF RAT
		GRAMS	WEEKS	GRAMS	GRAMS	
CASEIN	M	75	4	88	216	2.5
	M	82	4	80	219	2.7
	M	42	4	79	215	2.7
KING SALMON EGG PROTEIN	F	88	3	30	203	2.5
	M	69	4	110	225	2.0
	F	45	3	41	131	3.2

The data show that the rats on the diet containing salmon egg protein grew about as well as those fed casein. No gross symptoms of toxicity were manifested at the termination of the experiment. While it is realized that these experiments are not extensive, they do indicate that no acute toxicity resides in the defatted salmon egg meal and that the nutritional value appears to be very nearly equivalent to that of casein.

RECOVERY OF SALMON EGG FAT

The solvent in the acetone-water solution of salmon egg extractives was removed by distillation in a simple pot still at atmospheric pressure. The major part of the acetone was recovered by heating the mixture to 60° C. From all appearances this temperature was not measurably destructive to the lipide fraction which separated out as an oily layer. This oil layer was removed by decantation and subjected to further solvent removal at reduced pressure. The water phase was discarded after decantation. The final ethanol extract was also concentrated and the extractives added to the acetone soluble lipides. The resulting oil was a dark red color. In order to abstract some of the dark color and to refine the product further, the phospholipides were separated out by dissolving the oil in ether and adding acetone until no further precipitate formed. After precipitation of the lecithins by two repeated treatments with acetone, the solvent was removed from the oil fraction by distillation. The oil now possessed a light pinkish-red color which exhibited no tendency to darken upon standing. The acetone-precipitated sludge of phospholipides was freed of any residual oil by repeated extraction with acetone. Some darkening of the product, a light-brown greasy solid, occurred as the purification steps proceeded due undoubtedly to exposure to air during the processing steps.

TABLE 4 - PHOSPHOLIPIDE CONTENT OF SALMON EGGS1/

SPECIES OF SALMON	TOTAL EGG FAT2/	PHOSPHOLIPIDE3/ IN EGG	PHOSPHOLIPIDE4/ IN EGG	PHOSPHOLIPIDE5/ IN FAT
	PERCENT	PERCENT	PERCENT	PERCENT
PINK	35.0	13.6	11.7	33.4
RED	43.0	12.3	11.1	25.8
CHUM	26.5	12.9	10.4	39.2
COHO	38.0	15.3	12.4	32.6

1/ ALL VALUES ARE GIVEN ON A MOISTURE-FREE BASIS.
2/ ETHYL-ETHER SOLUBLE FAT.
3/ HALPERN (1945) SELECTIVE EXTRACTION METHOD.
4/ PHOSPHOROUS DETERMINATION, A.O.A.C. V, P. 21.
5/ BASED ON THE PHOSPHOROUS DETERMINATION.

The process of fat and phospholipide fractionation was applied to the solvent extractives from the eggs of four species of salmon. Yields of the separate fractions are given in Table 4.

As indicated in Table 4, the phospholipide fraction constitutes about one-third of the total fat. It appears that recovery and partial purification of the lecithin fraction of the extracted fat would be relatively simple.

DISCUSSION AND SUMMARY

One of the purposes of this investigation was to determine the possibility of utilizing salmon eggs from Alaskan salmon cannery waste for the production of cholesterol, lipide, and protein. Some of the references in the chemical literature held promise that salmon roe would prove to be an especially valuable source of cholesterol. Our observations have shown salmon eggs to be only average in cholesterol content, for example, approximately the same as hens' eggs. In view of these findings it appears unlikely that salmon eggs could be profitably processed for their cholesterol content alone. However, the protein, fat, and lecithin fractions prepared by solvent extraction of the raw eggs appear to be of high quality and to offer promise of economic recovery. The egg protein, judged on its appearance, odor, and preliminary nutritional evaluation appears worthy of further study. It is also possible that salmon egg protein may possess special properties desirable in certain industrial applications, such as the sizing of paper, manufacture of plastics, etc.

The salmon egg fat fractions, either combined or separated into glyceride and phospholipide portions, seem to be worthy of commercial exploitation. For example, because of the highly unsaturated nature of salmon egg oil (iodine number of about 220), it is believed that either directly or after slight modification, it would be suitable for incorporation into quick-drying paints and varnishes. The existing prices for oil, both edible and nonedible, and for commercial lecithin makes the recovery of these two materials from salmon eggs a promising possibility. With the fat content of salmon eggs ranging from 11 to 14 percent on the raw material basis, and with lecithin comprising about one-third of the total fat, the possibility of recovering these materials along with a high quality protein, seems to warrant further investigation.

ACKNOWLEDGMENT

Hugo W. Nilson of the Service's College Park Fishery Technological Laboratory carried out the animal feeding studies for the authors.

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FEEDING STUDIES WITH THE GUM OF GRACILLARIA CONFERVOIDES AND CARBOXYMETHYLCELLULOSE

By Hugo W. Nilson* and Maurice Bender*

ABSTRACT

GRACILLARIA GUM AND CARBOXYMETHYLCELLULOSE ARE WHOLESOME PRODUCTS WHEN FED TO RATS AND MICE IN COMPARATIVELY LARGE QUANTITIES FOR PERIODS FROM WEANING TO DEATH.

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INTRODUCTION

Investigation of the possibility of replacement of imported agar with seaweed gums of domestic origin, particularly for use as bacteriological media, was a wartime project of the Service's Fishery Technological Laboratory at College Park, Maryland. The gum of *Gracillaria confervoides* (North Carolina) met the specifications of the *Pharmacopoeia of the United States XIII* (1947) for agar. But, it was shown by Lee and Stoloff (1946) that this gum could not replace agar as a media for micro-organisms, at least for forensic purposes, since it exhibits a comparatively high degree of syneresis. The liquid of syneresis permitted spreading of plate cultures so no accurate counts could be made. The great majority of samples which were extracted also had such high viscosity at temperatures above 45° C. that they were unsuitable for bacteriological media involving mixing the inoculum with the medium.

However, the gum from *Gracillaria* could replace agar from *Gelidium* in certain industrial uses to good advantage. The difficulty in harvesting the seaweed has made the cost of material so high that postwar production, at least on any sizable scale, has not been continued. The data on feeding studies are reported herewith since domestic production may again be undertaken if more efficient harvesting and manufacturing processes can be devised.

Carboxymethylcellulose is made from non-fishery sources. The gum in solution, however, showed some initial promise of being used as a glaze on frozen fishery products. Unpublished data from this laboratory indicate that the carboxymethylcellulose film dries out and becomes brittle when fishery products, mostly whole fish which have been glazed with the solution, are stored in the freezer. This permits dehydration of the products. The experiments did not show sufficient promise to recommend this gum as a coating medium. The feeding studies are reported herewith since the gum is used in considerable quantities in the food industry.

ANIMAL FEEDING TESTS

Rats and mice were allotted to the experiment at about weaning age. They were housed individually in wire screen cages fitted with screen floors. Food and water were allowed *ad libitum*. Live weight and food consumption data were taken at weekly intervals. Only male rats were used in the tests with *Gracillaria* gum, but both sexes were used in the tests with carboxymethylcellulose.

Groups were fed the control diet and diets containing 5 and 10 percent *Gracillaria* gum and 5 percent carboxymethylcellulose. The control diet consisted of casein, 15; lactalbumin, 5; lard, 15; brewer's yeast, 5; wheat germ, 2; salt mixture, U.S.P. XII, No. 2 for vitamin A and D assay, 4; cod liver oil, 2; and

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WEIGHING A TEST ANIMAL AT THE COLLEGE PARK FISHERY TECHNOLOGICAL LABORATORY.

an equal mixture of sucrose and and corn starch dextrin, 52 parts by weight. The different gums were incorporated into this diet at the expense of an equal amount of the sucrose-dextrin mixture.

The Gracillaria gum was purchased on the open market (1944) and the two lots which were used analyzed, respectively, 13.33 and 14.73 percent moisture; 1.48 and 0.86 percent protein (N x 6.25); 7.95 and 5.32 percent ash; and 77.24 and 79.09 percent carbohydrates (by difference). This gum was produced commercially by various companies operating at Beaufort, North Carolina. The carboxymethylcellulose, low viscosity type, was supplied by the Hercules Powder Company of Wilmington, Delaware.

The initial test with two levels of Gracillaria gum was carried out for a 10-week period. Comparable data are given for the single level of carboxymethylcellulose which was fed. The data in Table 1 are comparable to those for algin reported by Nilson and Lemon (1942), and for agar and Irish moss reported by Nilson and Schaller (1941). The only statistically significant differences from the controls were greater mean food and water requirements per unit gain in weight for the group fed the 10 percent level of Gracillaria gum.

Table 1 -- Feeding data with Gracillaria gum and carboxymethylcellulose during the ten-week test with groups of 10 rats each

Diet designation	Mean daily gain	Coefficient of variation	Per-gram gain in weight: Mean food	Coefficient of variation	Mean water	Coefficient of variation
	grams	percent	grams	percent	milliliters	percent
Control	3.48	11	2.92	8	5.74	19
Gracillaria:						
5 percent	3.41	7	3.08	5	5.42	12
10 percent	3.35	12	3.21*	6	6.31	15
Carboxymethylcellulose:						
5 percent	3.26	18	3.16	13	no data	no data

*A significant difference from control according to the Fisher t test.

OBSERVATIONS ON ANIMAL FEEDING TESTS

The coefficient of variation of mean weekly food intake for the group of rats fed carboxymethylcellulose is nearly twice that of the groups fed Gracillaria gum. The greater variation apparently was due to what seemed to be diarrhea. The smeary feces were very characteristic but did not seem to be a symptom of an injurious process. It was most probably due to the low absorption quality of the purified diet. One litter of five rats was allotted to a group fed a mixture of 5 percent carboxymethylcellulose and 95 percent ground Purina Dog Chow by weight. These rats were fed the diet for a month. They did not grow as well as the rats fed the same gum in the purified diet, but the feces were firm in texture and well formed.

Gross necropsy studies of the rats fed the 10 percent level of Gracillaria gum at the close of the 10-week test did not reveal any pathology. The remain-

ing animals were fed the diets for a year (the rats fed the 5 percent level of carboxymethylcellulose), or until they died. The comparative data calculated for the 10-week period indicate that both gums were wholesome.

The data in Table 2 show that the various animals receiving the 5 percent levels of Gracillaria gum or carboxymethylcellulose lived as long as the respective control animals. Those which died and upon which a post-morten examination could be made did not show any gross symptoms characteristic of any toxic condition. The data in Table 3 indicate no statistically significant differences between groups in mean maximum weight, mean weekly intake of food, or mean weekly intake of water where comparisons are possible.

Table 2 - Length of life of animals fed Gracillaria gum and carboxymethylcellulose

Diet designation	Length of life in weeks*
Rats:	
Control	22, 51, 68, 70, 70, 82, 82, 105, 128
Gracillaria: 5 percent	11, 17, 58, 60, 78, 84, 86, 88, 93, 94
Carboxymethylcellulose: 5 percent	sacrificed after 51 to 57 weeks
Mice:	
Control	25, 35, 50, 51, 75
Carboxymethylcellulose: 5 percent	1, 20, 24, 27, 39, 39, 54, 55, 66, 77

*Only male rats were used in tests with Gracillaria gum. Both male and female animals were used in tests with carboxymethylcellulose.

Table 3 - Feeding date with Gracillaria gum and carboxymethylcellulose during long time tests

Diet designation	Number of animals	Mean maximum weight	Standard error of maximum weight	Mean weekly intake of gum	Mean weekly intake of basal diet	Standard error of total food consumption	Mean weekly water intake	Standard error of water intake
		grams	grams	grams	grams	grams	milliliters	milliliters
Rats:								
From weaning to death:								
Control	10	452	32	0.0	71.4	3.28	143.5	6.96
Gracillaria; 5 percent	10	418	30	3.5	67.4	2.73	145.4	4.15
For one year:								
Carboxymethylcellulose, 5 percent	10	404	19	3.9	73.6	3.20	-	-
Mice:								
From about weaning to death:								
Control	5	31	3	0.0	18.9	2.55	-	-
Carboxymethylcellulose, 5 percent	10	32	3	1.1	21.5	1.69	-	-

The rats fed the 5-percent level of carboxymethylcellulose were sacrificed after one year and the principal organs were examined microscopically. The pathologist found no evidence that the carboxymethylcellulose produced any specific uniform change in the heart, liver, kidney, spleen, or gastrointestinal tract. There was no evidence of gross pathology when the rats were sacrificed.

CONCLUSION

In summary, the data indicate that both the Gracillaria gum and carboxymethylcellulose are wholesome products when fed to rats and mice in comparatively large quantities for periods from weaning to death.

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STUDIES ON ANALYTICAL METHODS OF EXTRACTING VITAMIN A AND OIL FROM FISHERY PRODUCTS

PART IV - EXPERIMENTS ON THE EXTRACTION OF LOW-OIL-CONTENT LIVERS WITH ACETONE, ETHYL ETHER, AND PETROLEUM ETHER

By F. Bruce Sanford and William Clegg*

This report presents data on further experiments (Sanford and Karrick 1950) carried out with a view toward development of improved methods for extracting oil and vitamin A from low-oil-content fish livers. The rockfish (Sebastodes sp.) livers used in the experiments reported here were from the same batch employed in the earlier series.

Table 1 - The Apparent Concentration of Oil in Varied-Weight Samples of Low-Oil-Content Liver Determined By Means of the Shaking Method with Acetone as the Solvent[1]

Approximate weight of liver sample	Apparent concentration of oil in liver: Replicate sample number 1	2	3	4	Average of replicates
Grams	Percent	Percent	Percent	Percent	Percent
12	14.4	13.6	14.1	-	14.0
5	18.4	18.0	18.4	18.1	18.2
2	18.4	18.8	19.2	18.9	18.8

1/Fifty ml. of acetone was used. The shaking bottle had a capacity of 180 ml.

Two methods of oil extraction were studied: the shaking method and the soxhlet method. The equipment and procedure used in the soxhlet method were standard, except that powdered pumice was mixed with the liver material in the extraction thimble and raw, undried liver was used. All the extraction thimbles contained approximately the same weight of liver material (5.4 grams). Details of the shaking method were described in the earlier paper. The data are presented in Tables 1 to 4.

Table 2 - Data Obtained by Soxhlet Extracting Low-Oil-Content Liver with Acetone and Subsequently Purifying the Extractives with Acetone, Ethyl Ether, and Petroleum Ether

Step	Procedure	Apparent concentration of oil in liver: Replicate sample number 1	2	3	4	5	6	Average of replicates
		Percent	Percent	Percent	Percent	Percent	Percent	Percent
A	Soxhlet extraction for 16 hours with acetone	28.6	27.8	28.0	28.4	29.4	28.0	28.4
B[1]	Acetone purification of extractives from step A	21.1	20.3	19.4	19.4	21.0	20.9	20.4
C	Acetone purification of extractives from step B	20.9	20.1	19.3	19.2	20.5	20.5	20.1
D[2]	Ethyl ether purification of extractives from step C	19.2	19.0	18.9	19.0	19.1	19.2	19.1
E	Ethyl ether purification of extractives from step D	19.0	18.8	18.6	18.9	18.9	19.0	18.9
F[2]	Petroleum ether purification of extractives from step E	17.4	17.2	17.0	17.4	17.4	17.4	17.3
G	Petroleum ether purification of extractives from step F	17.3	17.1	17.0	17.4	17.2	17.3	17.2

1/After the acetone used in the original soxhlet extraction had been evaporated from the extraction flask and the weight of extractives determined, the soluble portion of the extractives was redissolved in added acetone, and the resulting solution was freed of undissolved residue by passing the solution through a fritted glass filter funnel. The solvent was then evaporated and the weight of extractives determined. The purification steps that follow were carried out in the same manner, using the solvent designated in that particular step. The acetone-insoluble residue from step B was soluble in hot water.
2/The residue was soluble in 95-percent ethanol.

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Table 3 - Data Obtained by Soxhlet Extracting Low-Oil-Content Liver with Ethyl Ether and Subsequently Purifying the Extractives with Ethyl Ether and Petroleum Ether

Step	Procedure	Apparent concentration of oil in sample						
		Replicate sample number						Average of replicates
		1	2	3	4	5	6	
		Percent	Percent	Percent	Percent	Percent	Percent	Percent
A	Soxhlet extraction for 16 hours with ethyl ether	18.4	18.0	18.3	18.0	19.0	18.4	18.4
B	Ethyl ether purification of extractives from step A	18.2	17.8	18.1	17.8	18.7	18.1	18.1
C	Ethyl ether purification of extractives from step B	18.1	17.8	18.0	17.8	18.6	18.0	18.0
D	Petroleum ether purification of extractives from step C	17.3	16.8	17.2	17.0	17.3	17.0	17.1
E	Petroleum ether purification of extractives from step D	17.2	16.8	17.1	17.0	17.3	16.6	17.0

It was found that:

1. In the extraction of low-oil-content liver by means of the shaking method and the use of acetone (without dispersing or drying agents), relatively more extractives were obtained from small-size samples than from those of large size (Table 1).

2. In the soxhlet extraction of low-oil-content liver for 16 hours with acetone, certain materials were extracted that were not readily soluble in acetone but were readily soluble in hot water (Table 2, footnote 1).

3. In soxhlet extracting of low-oil-content liver for 16 hours with acetone and then purifying the extractives with acetone, ethyl ether, and petroleum ether, certain of the extractives that were readily soluble in acetone did not dissolve in ethyl ether; and certain of the remaining extractives that were readily soluble in ethyl ether did not dissolve in petroleum ether. The acetone-soluble residues that were insoluble in ethyl ether dissolved in 95-percent alcohol, as did also the residues that were soluble in ethyl ether but insoluble in petroleum ether (Table 2, footnote 2).

Table 4 - Data Obtained by Soxhlet Extracting Low-Oil-Content Liver with Petroleum Ether and Subsequently Purifying the Extractives with Petroleum Ether

Step	Procedure	Apparent concentration of oil in liver						
		Replicate sample number						Average of replicates
		1	2	3	4	5	6	
	<u>Experiment 1</u>	Percent	Percent	Percent	Percent	Percent	Percent	Percent
A1/	Soxhlet extraction for 16 hours with petroleum ether	3.1	10.6	6.2	2.8	4.1	6.6	5.6
	<u>Experiment 2</u>							
A	Soxhlet extraction for 16 hours with petroleum ether	12.1	15.4	13.8	9.8	12.2	13.7	12.8
B	Petroleum ether purification of extractives from step A	11.9	15.2	13.7	9.5	12.1	13.1	12.6
C	Petroleum ether purification of extractives from step B	11.9	15.3	13.8	9.5	12.2	13.1	12.6

1/Pumice was not mixed with the liver material in the extraction thimble.

4. Using the soxhlet method, more ethyl ether or petroleum ether solubles were obtained when the initial soxhlet extraction was made with acetone than when it was made with ethyl ether or petroleum ether. Likewise, more petroleum-ether solubles were obtained when the initial extraction was made with ethyl ether than when it was made with petroleum ether (Tables 2, 3, and 4).

5. In the soxhlet extraction of low-oil-content liver, using petroleum ether as the solvent, the mixing of powdered pumice with the liver sample in the extraction thimble appeared to aid extraction (Table 4).

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NOTE: THE OTHER PARTS OF THIS PAPER APPEARED AS FOLLOWS: PART I - "VITAMIN A POTENCIES OF OIL FROM GRAYFISH LIVERS OBTAINED BY EXTRACTION WITH PETROLEUM ETHER AND BY COOKING WITH WATER," BY D. MIYAUCHI AND F. B. SANFORD, COMMERCIAL FISHERIES REVIEW, SEPTEMBER 1947, VOL. 9, NO. 9, AND ALSO AS SEPARATE NO. 186; PART II - "EXPERIMENTS ON THE SOLVENT EXTRACTION OF LOW-FAT LIVERS, SAME REVIEW, FEBRUARY 1949, VOL. 11, NO. 2, AND ALSO AS SEPARATE NO. 224.

FREEZING AND CANNING KING CRAB

The techniques used in the preparation and handling of king crab are of primary importance in maintaining the quality of the canned or frozen product. King crab meat must be processed with utmost care to insure the maximum retention of color, flavor, and texture. A high quality product can be obtained only if careful attention is given to initial phases of handling the king crab, such as holding the live crab, butchering, cooking, cooling, removing the meat, and cleaning. Recommendations are based on observations of experimental and commercial packs.

Additional factors pertaining to packaging of meat for freezing and to heat processing are discussed in this publication.

By John A. Dassow

--Fishery Leaflet 374 (May 1950)

A CHEMICAL EVALUATION OF TUNA-LIVER AND BEEF-LIVER MEALS PREPARED BY DIFFERENT METHODS

By G. Ivor Jones* and William H. Hoyer**

ABSTRACT

MEALS PREPARED FROM BEEF LIVER, ALBACORE TUNA LIVER, AND YELLOWFIN TUNA LIVER BY THE PROCESS OF LYOPHILIZATION RETAINED THE GREATEST AMOUNT OF THE THIAMINE, NIACIN, AND RIBOFLAVIN PRESENT IN THE RAW LIVER.

DEHYDRATION OF THE RAW LIVER BY MEANS OF REPEATED EXTRACTION WITH ACETONE CAUSED CONSIDERABLE LOSS OF THE THREE VITAMINS STUDIED. ACETONE, HOWEVER, REMOVED MOST OF THE FAT WHICH IS EASILY OXIDIZABLE AND, THEREFORE, OBJECTIONABLE WHEN PRESENT IN THE FINISHED MEAL.

VACUUM DRYING OF THE RAW LIVER AT 100° F. PRODUCED DARK-COLORED MEALS WHICH RETAINED APPRECIABLE AMOUNTS OF THIAMINE, NIACIN, AND RIBOFLAVIN. THIAMINE WAS THE MOST EASILY DESTROYED OF THE THREE VITAMINS INVESTIGATED. MEAL COULD NOT BE SUCCESSFULLY PREPARED FROM ALBACORE TUNA LIVER BY THIS PROCESS BECAUSE OF THE PRESENCE OF A LARGE AMOUNT OF FAT.

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INTRODUCTION

As part of a continuing project on the utilization of wastes of the fishing and fish-canning industry, a survey of tuna canning plants in the latter part of 1948 revealed a large quantity of yellowfin and albacore tuna livers available in storage with little market value for production of vitamin A oil. Since reduction of the tuna livers to commercial fish meal would yield only the minimum potential value of the raw material, it was decided to investigate the possibility of preparing high-grade meals by several different methods. The meals could then be evaluated as to possible use as supplemental feeding materials for fish hatcheries, animal and poultry nutrition, or for isolation of valuable chemical substances. In the present report, the preparation of the various meals by different methods are discussed in detail, followed by the results of proximate chemical analysis and data on three members of the vitamin B complex, namely: thiamine, niacin, and riboflavin. Biological evaluation of the raw tuna liver in the feeding of hatchery fish will be presented in another report considering the nutritional value of many raw natural foods for the propagation of hatchery-reared red or sockeye salmon. Comparison of the raw tuna livers and meals with that of beef liver was decided upon since the latter is a standard article of commerce whose value in nutrition of hatchery fish has been established over the years by numerous investigations.

EXPERIMENTAL PROCEDURES

The frozen raw beef liver was obtained from the regular supply of "fluky" beef liver used in the production hatchery of the Fish and Wildlife Service at

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Leavenworth, Washington. The frozen raw tuna livers were furnished through the courtesy of the Columbia River Packers Association of Astoria, Oregon. Neither the size of the fish nor the geographical location of capture are known, as the livers were received in five-gallon tin cans. Upon its receipt in December 1948, the frozen liver material was chopped into smaller chunks while still semi-frozen and then passed through a meat grinder having a plate with 1/8-inch holes. The ground material was thoroughly mixed before drawing samples for analyses of the raw liver and for the preparation of meal. Samples were packaged in one-gallon fibreboard cartons, frozen, and held at 0° F. until used.

Three types of meals were prepared from the raw liver. The methods used included "lyophilization" or vacuum-freeze drying, vacuum drying at 100° F., and dehydration by repeated extraction with acetone. It was believed that the preparation of a meal by vacuum drying of the ground livers while frozen would be least destructive of the essential vitamins and other nutritional elements. Vacuum drying at 100° F. in a commercial-type steam-jacketed dryer would undoubtedly cause some nutritional loss but would be expected to produce a meal superior to those prepared in conventional direct-flame fish-meal dryers. Dehydration by repeated extraction with acetone would have the advantage of preparing a meal without the application of heat, and in addition reduce the fat content to a lower level, which would be especially advantageous with livers having a high fat content. However, it is quite possible that this method would remove a portion of the water-soluble B complex vitamins which would, of course, be a distinct disadvantage.

PREPARATION OF LYOPHILIZED MEAL: In preparing the lyophilized meal, the frozen ground liver samples were thawed at room temperature and then further blended and mixed with one-half their volume of distilled water in a Waring Blendor. Approximately 600 ml. of this mixture was placed in a 3-liter round-bottom distilling flask equipped with a standard-taper glass connector. The material was frozen in a thin layer on the inside surface of the flask by alternately rotating it and then immersing it in a mixture of dry ice and alcohol. After the samples were frozen, the flasks were connected to a vacuum system containing a condenser immersed in a bath consisting of dry ice and alcohol. The contents of the flask were dry in approximately 12 hours. The meals prepared by this method were vacuum-packed in 1/2-pound flat, hermetically-sealed cans and held at 0° F. for subsequent analysis.

PREPARATION OF 100° F. VACUUM-DRIED MEAL: Preparation of a liver meal vacuum dried at 100° F. was accomplished by charging 40 to 50 pounds of the thawed-ground liver sample into a small Stokes horizontal, rotary-paddle, steam-jacketed, vacuum dryer. The charge was maintained at a temperature of 100° F. by admitting steam at a pressure of 5 to 10 pounds into the external jacket of the dryer and by maintaining a vacuum of 27 inches in the drying chamber. Some difficulty was encountered with all three types of livers. Beef liver and yellowfin tuna liver had a tendency for forming into round balls. Midway during the drying process it was found expedient to break up these balls of semidried liver in order to hasten drying. The difficulties might have been overcome by changing the technique but, since our interest was in the meals and not primarily in the processing methods, no attempt was made to improve the procedure.

Albacore livers did not yield a satisfactory product by this method. The fat content of these livers is relatively high (about 18 percent fat in raw liver) and, under the conditions existing in the dryer, produced a sticky mixture with the appearance of grease. Since it did not appear that a satisfactory albacore

meal could be readily prepared by this method, this part of the experiment was abandoned.

After drying the beef and yellowfin tuna liver in the Stokes vacuum dryer, the residue was finely ground and packed into 1/2-pound flat cans, vacuum-sealed, and held at 0° F. storage until analyzed.

PREPARATION OF ACETONE DEHYDRATED MEAL: Dehydration of the thawed, ground, raw liver material with acetone was carried out by covering 3 kg. of the liver with 12 liters of C. P. acetone in a large glass jar fitted with a plywood cover. The mixture was stirred intermittently during the extraction process, which was allowed to proceed for 8 hours. At this time, the mixture was allowed to settle and the supernatant liquid was siphoned off without disturbing the solid residue. The residue was then covered with a second portion of acetone. A total of 3 extractions were made. The third acetone extraction appeared to remove only a trace of color from the liver solids and it was surmised that the moisture content of the residue had by then been reduced to less than 10 percent, which later proved to be correct upon analysis. The last traces of acetone were removed from the solids by air drying before a fan in a well-ventilated room. When dry, the powdered meal was vacuum-packed in tin containers and held at 0° F. during storage.

ANALYTICAL DETERMINATIONS: Determination of moisture, total nitrogen, fat, and ash on both the raw livers and the prepared meals were made using modifications of the A.O.A.C., VI (1945) methods of analysis. These modifications have been developed at the Seattle laboratory after many years of study on analytical methods applicable to fish products and by-products. The modified procedures are as follows:

Moisture: Weigh 5 to 10 g. of sample into tared aluminum dishes provided with covers. For wet material, mix sample with about 20 g. of purified sand. Place dishes in an air oven at 115° C. for 3½ hrs., or in a vacuum oven (1 mm. mercury or less) at 80° C. for 5 hrs. Cool samples in a desiccator for 1 hr. and weigh. Return samples to oven for 30 min., cool, and weigh to determine if weight is constant.

Total Nitrogen: Weigh 0.5 to 2.5 g. of sample (percent of moisture governs amount) into clean, glass cells. Transfer cells to Kjeldahl flasks. Add 6 to 8 glass beads, 10 g. of anhydrous Na_2SO_4 and a granule of selenium catalyst. Finally, add 25 ml. of concentrated H_2SO_4 and digest the mixture until it becomes clear, plus an additional 30 to 60 min. to assure complete digestion. Cool the digested mixture and add 180 to 200 ml. of distilled water. Place 25 ml. of 0.5 N HCl plus 100 ml. distilled water, plus 4 drops of indicator composed of 0.2-percent methyl-red and 0.1-percent methylene-blue in the receiving flask. Add a few drops of phenolphthalein indicator to the digest. Pour carefully into the tilted flask about 100 ml. of 45-percent NaOH solution. Immediately connect flask to the distillation assembly. Distill 150 ml. volume or until "bumping" begins. Add an additional 4 drops of the indicator described above to the receiving flask to enhance the end point during titration. Total nitrogen multiplied by factor 6.25 was used to calculate the value for protein.

Fat: Weigh 4 to 6 g. of sample into tared alundum thimbles, cover sample with a thin layer of cotton and extract for 16 hrs. with 35 ml. of ethyl ether in a Bailey-Walker extractor.

Wet materials, such as raw liver, are weighed on several grams of dry pumice powder placed in a tared thimble. After weighing, the sample and pumice are mixed. The pumice prevents seepage of water through the pores of the thimble.

The surplus solvent in the extract is distilled off to a low volume, and the last traces are removed on a hot plate at low heat. Place flasks in a vacuum oven at 80° C. with a vacuum of 24 to 25 in. for 1 hr. Cool in a desiccator for 45 min. and weigh.

Ash: Weigh 3 to 4 g. of sample into tared crucibles. Dry samples in an air oven at 80° C. for 2 hrs. Carbonize samples carefully over an open flame, then place in an electrically-heated muffle at a temperature of 550° C. for 4 hrs. Cool in a desiccator and weigh.

Thiamine: Determination of this vitamin in the raw and processed samples was carried out using the thiochrome method described in Methods of Vitamin Assay of The Association of Vitamin Chemists (1947).

Niacin and Riboflavin: These two vitamins were determined using the microbiological procedures of Roberts and Snell (1946).

ANALYTICAL RESULTS: The results of the proximate analyses of livers and liver meals of beef, albacore tuna, and yellowfin tuna are presented in Table 1. The values are averages of 3 or more replicates in all cases except where an individual replicate value was at large variance from the others, in which event it was discarded. No special difficulty was experienced in making any of the determinations. It was noted, however, that in titrating the ammonia distillate in the determination of total nitrogen, a sharper end point was obtained when an indicator mixture composed of equal parts of 0.2-percent methyl-red and 0.1-percent methylene-blue in ethanol was used in place of the straight methyl-red indicator. The color change of this mixture began as a red-violet at pH 5.2 with a change to a grey-blue at pH 5.4 and finally to a green at pH 5.6, which was taken as the correct end point.

Table 1 - Proximate Analyses1/ of Livers and Liver Meals of Beef, Albacore Tuna, and Yellowfin Tuna

Type of Liver	Condition	Moisture	Solids	Protein	Fat	Ash
		%	%	%	%	%
Beef	Raw	68.4	31.6	18.9	9.3	1.3
Beef	"Lyophilized"	4.8	95.2	61.2	18.8	4.2
Beef	Vacuum-dried at 100° F.	3.5	96.5	61.6	20.5	4.2
Beef	Acetone dehydrated	6.7	93.3	75.4	4.6	3.7
Albacore tuna	Raw	60.3	39.7	16.6	18.5	1.1
Albacore tuna	"Lyophilized"	3.9	96.1	39.4	50.8	2.5
Albacore tuna	Acetone dehydrated	9.2	90.8	73.0	4.8	4.4
Yellowfin tuna	Raw	69.8	30.2	23.2	3.1	1.5
Yellowfin tuna	"Lyophilized"	4.6	95.4	72.0	11.6	4.6
Yellowfin tuna	Vacuum-dried at 100° F.	2.9	97.1	72.3	10.7	4.9
Yellowfin tuna	Acetone dehydrated	9.0	91.0	79.8	2.1	5.0

1/ Values reported represent an average of 3 to 6 replicates.

The results of the vitamin analyses are presented in Table 2. The thiamine values represent an average of duplicate samples. Several confirmatory assays

were made in order to check some questionable values. Recovery experiments indicated that 95 percent recovery could be expected. A standard thiamine sample was carried through all the steps of the determination each time that samples were assayed.

DISCUSSION OF RESULTS: Meals prepared by drying in a vacuum at 100° F. had the lowest moisture content (Table 1). Drying by lyophilization produced a meal nearly as low in moisture content. Undoubtedly, the moisture content of the lyophilized meals could have been reduced still further by allowing the process to continue for a longer period of time. The acetone-dehydrated and partially-defatted meals had a higher moisture level than those prepared by the other two methods, but was still under 10 percent moisture. All of these meals had apparently good storage qualities, except that the fat present, especially in the lyophilized albacore tuna-liver meal, oxidized rapidly resulting in a rancid-smelling product. This meal darkened rapidly when exposed to air.

The meals appeared to have no tendency to pick up moisture on exposure to air at room temperature nor was there any tendency of the material to mold or to spoil.

The high fat content of lyophilized albacore tuna-liver meal is objectionable in that it would reduce the storage life of the finished product and may be undesirable in the diet of young hatchery fish. However, the recovery of this fat, either from the raw liver during meal preparation or by extraction from the dried meal, would seem to pose no insurmountable problems, and the oil so obtained might even be of considerable economic value.

In order to compare the effect of the various drying methods on the vitamin content of the finished meals, these values were calculated on the moisture-free basis and are presented in Table 2. It will be noted that dehydration by means of lyophilization caused no significant loss of thiamine in the beef-liver meal. The thiamine content of the lyophilized albacore and yellowfin tuna-liver meals is approximately twice that present in the raw material when compared on the moisture-free basis. These seemingly irreconcilable data can best be explained by the fact that the thiamine and other vitamin assays were not performed on either the raw material or the several meals until all of the meals had been prepared. The lyophilized meals were prepared first, early in the 6-month period. Thus, the raw-ground liver had been held in frozen storage at 0° F. for about 6 months before the vitamin determinations were made. The reason for the values of thiamine in the raw material being lower than in the lyophilized meal can best be explained by destruction of the vitamin due to liberation or excitation of enzyme systems in the raw liver caused by grinding prior to storage. The data in Table 2 indicate the lyophilized yellowfin tuna-liver meal to be the best source of the 3 vitamins tested. The liver meals prepared by vacuum drying at 100° F. appear to retain niacin and riboflavin without any appreciable destruction, but they do sustain a considerable loss of thiamine amounting to approximately 50 percent loss in the beef-liver meal and about 66 percent in the yellowfin tuna-liver meal.

Dehydration of the raw liver by means of acetone causes nearly total loss of thiamine in the tuna-liver meals. Acetone extraction also removed one-half to two-thirds of the niacin and riboflavin content. This method of meal preparation produces a light-colored meal, low in fat, but undoubtedly removes, to some degree, members of the vitamin B complex present which, of course, disturbs the nutritional balance of the final product.

Table 2 - Thiamine, Niacin, and Riboflavin Content of Livers and Liver Meals of Beef, Albacore Tuna, and Yellowfin Tuna

Type of Liver	Condition	Moist Basis			Moisture-free Basis		
		Thiamine	Niacin	Riboflavin	Thiamine	Niacin	Riboflavin
		.... (Micrograms per gram)					
Beef	Raw	2.8	99	20	8.9	313	63
Beef	"Lyophilized"	8.6	365	87	9.0	383	91
Beef	Vacuum-dried at 100°F.	4.6	423	94	4.8	438	97
Beef	Acetone dehydrated	3.5	77	65	3.8	83	70
Albacore tuna	Raw	0.3	48	16	0.8	121	40
Albacore tuna	"Lyophilized"	1.4	110	25	1.5	115	26
Albacore tuna	Acetone dehydrated	0.2	39	32	0.2	43	35
Yellowfin tuna	Raw	0.9	83	19	3.0	275	63
Yellowfin tuna	"Lyophilized"	5.7	262	78	6.0	275	82
Yellowfin tuna	Vacuum-dried at 100°F.	2.0	269	67	2.1	277	69
Yellowfin tuna	Acetone dehydrated	0.2	28	43	0.2	31	47

SUMMARY

Meals of good appearance and apparently good keeping qualities can be prepared by lyophilization, vacuum drying at 100° F., and by dehydration by repeated extraction with acetone. Lyophilization caused the least destruction of nutritive components. Dehydration by means of acetone resulted in very appreciable loss of thiamine, niacin, and riboflavin in the dried meal, but this method possessed the advantage of removing most of the fat which in tuna livers is highly susceptible to oxidation. Great difficulty was encountered in attempting to prepare a meal from albacore livers by vacuum drying at 100° F. due to the large amount of fat present. Although the tuna-liver meals do not contain as much thiamine, niacin, or riboflavin as does beef-liver meal, it appears possible that by furnishing a higher proportion of them in the diet than is customary with beef liver, they might supplant the latter in the feeding of hatchery fish. It also seems possible that a good-quality tuna-liver meal would possess nutritive factors valuable as a vitamin supplement in animal and poultry feeds.

CONCLUSIONS

(1) Meals prepared from beef liver, albacore tuna liver, and yellowfin tuna liver by the process of lyophilization retain the greatest amount of the thiamine, niacin, and riboflavin present in the raw liver.

(2) Dehydration of the raw liver by means of repeated extraction with acetone causes considerable loss of the three vitamins studied. Acetone, however, removes most of the fat which was easily oxidizable and, therefore, objectionable when present in the finished meal.

(3) Vacuum drying of the raw liver at 100° F. produces dark-colored meals which retain appreciable amounts of thiamine, niacin, and riboflavin. Thiamine is the most easily destroyed of the three vitamins investigated. Meal could not be successfully prepared from albacore tuna liver by this process because of the presence of a large amount of fat.

ACKNOWLEDGEMENT

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UTILIZATION OF FISHERY BYPRODUCTS IN WASHINGTON AND OREGON

Very little fish scrap is being discarded in the states of Washington and Oregon. The small amount not utilized is either in an area where the supply is inadequate to support a commercial operation, or else the material is of such a nature that it does not command a market. Companies have failed because the supply of waste has been insufficient. Others have lost money on the production of materials not in demand. Anyone who intends to enter the field of byproducts should, therefore, make a thorough survey of the source of supply and the market for the finished product.

The byproducts industry is not static. Changes are taking place, and the field is becoming increasingly competitive. Fish waste, in earlier years, was thrown away. Later, it was utilized only by reduction plants. Now it is in demand for reduction purposes and for mink feed and other uses. With few exceptions, the operations have not produced appreciable revenue, and many firms have operated largely on a marginal basis. For this reason, there is a continuing and increasing pressure to find more remunerative uses for the waste. The problems to be solved are not easy; but with rapid acceleration in technological knowledge and the demands of a growing population, further changes are inevitable.

By F. Bruce Sanford

--Fishery Leaflet 370 (March 1950)

TECHNICAL NOTE NO. 5 -- "PINK YEAST" ISOLATED FROM OYSTERS GROWS AT TEMPERATURES BELOW FREEZING

In certain freezing tests carried out at the Service's College Park Fishery Technological Laboratory, several packages of oysters stored at 0° F. for a period of one month showed, upon thawing, a decidedly pink-colored liquor and pink-to-red spots on the oysters. There had been no signs of discoloration of the fresh oysters or liquor when first packaged. "Pink yeast" was suspected as being the causative agent for the discoloration.

Six oysters and a small amount of liquor were taken from each experimental lot and were macerated for two or three minutes in a Waring Blendor. A loopful of substance was streaked on Sabouraud's agar media in a Petri dish. Gram staining in each case revealed a mixed culture of yeast cells and a gram-positive rod bacterium. The growth of the bacterium was confluent and rapid, covering the yeast colonies. In order to inhibit the growth of the bacterium and make it possible to obtain a pure culture of the "pink yeast," one drop of 50 percent lactic acid was added to 10 ml. of Sabouraud's agar media. A culture of "pink yeast" was obtained and an isolated colony from the Sabouraud's agar media was transferred to a test tube of Sabouraud's broth media. This culture was used for the various inoculations made throughout this study.

It hardly seemed likely that growth would occur at as low a temperature as 0° F.; however, growth did take place in broth tubes at temperatures of 0° F., -14° F., and -30° F. to -35° F. Sabouraud's agar media in plates were also streaked with the isolated culture and the plates were stored for one to two months at 0° F. Small colorless colonies were noted on the media, after the frost had thawed from the inside of the plate. It is felt that colonies of this size could not possibly have developed during the half-hour thawing period. Rapid growth with pigment formation took place later at room temperature.

Individual oysters plus liquor were placed in sterile test tubes, inoculated with one of the yeast cultures, and stored for one month at 0° F. Samples of oysters which were not inoculated served as controls. After storage, one frozen oyster had four large pink colonies on the surface. Diffused pink-colored areas were on the surfaces of the other oysters. No visible growth of "pink yeast" was noted in the control samples. The colony formation, in particular, was considered good proof of growth at 0° F.

The inoculated tubes of Sabouraud's broth media which were stored at 0° F., -14° F., and -30° F. to -35° F., respectively, all showed small pink colonies in the frozen media, which indicated that growth had occurred at these temperatures.

From the foregoing results, it was concluded that "pink yeast" is capable of growing at temperatures of 0° F. to -30° F.

--Grace McCormack, Bacteriologist,
Fishery Technological Laboratory,
College Park, Maryland.

TECHNICAL NOTE NO. 6--VITAMIN-A POTENCIES OF LIVER OILS OF BERING SEA COD AND FLOUNDER

Oil and vitamin-A analyses were made of the livers of certain species of Bering Sea cod and flounder. The fish were obtained by the Exploratory Fishing and Gear Development Section of the Branch of Commercial Fisheries while conducting exploratory fishing in the northern Bering Sea area during June-July 1949 (Ellson, Powell, and Hildebrand 1950). The livers of the following species of fish were analyzed: Pacific cod (Gadus macrocephalus), flathead sole or flounder (Hippoglossoides robustus and H. elassodon), lemon sole or Alaska plaice (Pleuronectes quadrituberculatus), rock sole or flounder (Lepidopsetta bilineata), and yellowfin sole or mud dab (Limanda aspera).

Table 1 - Data on the Type of Fish, Sex, and Number of Fish Examined, and Average Value of the Measurements Obtained on Fish Length, Fish Weight, Liver Weight, Oil Concentration in Liver, and Vitamin-A Potency of Liver Oil

Species of fish	Sex of fish	Number of specimens	Fish length 1/		Fish weight		Liver weight		Oil concentration in liver	Vitamin-A potency of liver oil
		Number	Centimeters	Inches	Grams	Pounds	Grams	Pounds	Percent	"Spec" units per gram 2/
Pacific cod	Male	19	68.1	26.8	3,305	7.28	119	0.262	29.5	7,600
	Female	27	73.2	28.8	3,975	8.77	149	0.329	27.9	18,100
Flathead sole	Male	3	43.6	17.2	849	1.87	12	0.026	27.2	14,400
	Female	19	43.6	17.2	936	2.06	11	0.024	10.9	35,800
Lemon sole	Male	42	36.8	14.5	643	1.42	8	0.018	9.[illegible]	10,200
	Female	58	44.0	17.3	1,143	2.52	22	0.048	6.2	7,800
Rock sole	Female	7	43.5	17.1	1,281	2.82	13	0.029	3.3	37,000
Yellowfin sole	Male	2	36.1	14.2	495	1.09	5	0.011	9.3	10,600
	Female	100	39.3	15.5	704	1.55	12	0.026	5.1	20,200

1/Fish length was the distance from the tip of the nose to the fork of the tail.
2/2,000 x E (1 percent, 1 cm., 328 mmu., isopropanol, whole oil).

The livers were analyzed by the "shaking method," using ethyl ether as the solvent (Anonymous 1947). The data are reported in Tables 1 and 2.

The livers of the flathead sole, lemon sole, rock sole, and yellowfin sole were, on the average, less than an ounce in weight and contained only a small amount of oil, which was of a relatively low vitamin-A potency. Such livers are of only marginal value.

Oil concentrations and vitamin-A potencies of the cod livers reported here are similar to those of livers taken commercially in 1947 from fish caught in the Bering Sea off the Alaska Peninsula (Sanford and Nilson 1949). Northern Bering Sea cod livers may, therefore, be of potential commercial value. This conclusion, however, is contingent upon the price of vitamin A rising at least to the 1947 level.

Table 2 - Data on the Lowest and Highest Values Obtained in the Measurement of Fish Length, Fish Weight, Liver Weight, Oil Concentration in Liver, and Vitamin-A Potency of Liver Oil

Species of fish	Sex of fish	Fish length 1/		Fish weight		Liver weight		Oil concentration in liver		Vitamin-A potency of liver oil	
		Low	High	Low	High	Low	High	Low	High	Low	High
		Centimeters	Centimeters	Grams	Grams	Grams	Grams	Percent	Percent	"Spec" units per gram 2/	"Spec" units per gram 2/
Pacific cod	Male	56.8	79.0	2,010	5,840	52	210	10.3	52.8	1,500	18,400
	Female	62.5	87.0	2,240	7,825	47	288	1.2	51.5	1,100	73,100
Flathead sole	Male	42.2	44.9	782	894	10	13	20.2	36.9	6,120	26,700
	Female	39.5	48.0	652	1,750	7	16	4.2	29.0	7,600	93,600
Lemon sole	Male	30.3	42.4	366	910	3	15	3.8	34.8	865	102,000
	Female	34.2	55.9	580	2,100	3	42	1.4	17.2	1,840	42,300
Rock sole	Female	41.2	47.7	820	2,780	5	32	2.6	5.0	24,200	61,200
Yellowfin sole	Male	36.0	36.2	448	542	3	7	7.6	11.0	8,280	13,000
	Female	31.3	48.0	324	1,245	3	44	2.0	16.6	1,740	96,800

1/Fish length was the distance from the tip of the nose to the fork of the tail.
2/2,000 x e (1 percent, 1 cm., 328 mmu., isopropanol, whole oil).

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--F. Bruce Sanford, Chemist,
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INFORMATION SOURCES FOR STUDENTS OF COMMERCIAL FISHERIES

Fishery Leaflet 362, Information Sources for Students of Commercial Fisheries, is a 20-page publication designed to show the student various means of obtaining information on the commercial fisheries of North America. It is not meant to be a complete bibliography--only the principal contributions or bibliographies are listed.

References in this publication are listed under the following major categories: Agar; byproducts; canning; cookery; directories; employment; fish and fisheries; freezing; gear; libraries; marketing; oils, rancidity, antioxidants; salting; sanitation; smoking; spoilage; statistics; technical journals; trade journals; visual aids; and vitamin oils. Items which are recommended as basic sources are indicated.

By R. Paul Elliott

--Fishery Leaflet 362 (January 1950)

TECHNICAL NOTE NO. 7--RESULTS OF SOME TESTS WITH FROZEN LOBSTERS AND LOBSTER MEAT

INTRODUCTION

Very little information is available regarding the freezing characteristics of lobsters and lobster meat. Although the quantity available for freezing would undoubtedly be quite limited due to the heavy demand for live lobsters, it may be desired at times to freeze this product on a small scale. This is evident by the inquiries on freezing of lobsters received by the Fish and Wildlife Service from locker plant operators, and manufacturers and owners of home freezers.

With this in mind, a limited study of the storage of frozen lobsters and lobster meat was made. Since the work was restricted to only a comparatively small number of samples held at only one storage temperature, it should not be construed that the results are conclusive. Possibly other methods of preparation and lower storage temperatures would alter the findings considerably.

PREPARATION OF SAMPLES

Live lobsters and freshly-cooked lobster meat were obtained and prepared for the tests in Gloucester, Mass. Five lots were prepared for freezing, as follows:

1. Whole live lobsters. These are designated later in this report as the "uncooked" sample.

2. Whole cooked lobsters. These were live lobsters boiled for 16 minutes in salted water (1 tablespoon of salt to 1 quart of water).

3. Whole cooked lobsters. Similar to (2), which were frozen in blocks of ice after one month of storage at 0° F.

4. Cooked lobster meat packed without liquid in half-pound tin cans and hermetically sealed.

5. Cooked lobster meat packed in $1\frac{1}{2}$ percent brine in half-pound tin cans and hermetically sealed.

All samples were frozen in circulating brine at a temperature of approximately 0° F., followed by storage in a room at the same temperature. The whole lobsters were given an ice glaze to retard desiccation.

It had been planned to freeze several cooked lobsters in blocks of ice at the time the samples were being prepared in order to ascertain whether a minimum of desiccation during storage would prevent changes in the texture of the meat. Because of certain difficulties this was not done at the time of freezing but was done a month later (Lot No. 3). The samples were kept ice-glazed during this interval.

All of the lots were shipped with dry ice in an insulated container to the Service's Fishery Technological Laboratory in College Park, Md., and were received in a frozen condition. The whole lobsters were then reglazed and packaged individually in heavy metal foil wrappers. All samples were held in storage at 0° F.

At monthly intervals, samples from each lot were removed from frozen storage and allowed to thaw at room temperature. Those that were uncooked were thawed, then boiled in salted water for 16 minutes, and cooled before being tested. The meat was picked from the whole lobsters and cut into pieces for palatability tests. The meat from fresh-cooked live lobsters was used as controls. All samples were designated by a code unknown to the judges at the time of testing. Members of the laboratory staff, accustomed to making taste tests, served as judges. Scores were based on the appearance, flavor, and texture of the lobster meat. A score of 85 or over is considered satisfactory.

RESULTS AND DISCUSSION

The palatability scores obtained in the tests are given in Table 1. The frozen uncooked lobsters consistently received a satisfactory and relatively high score at each examination during the six-month period. The flavor and texture of the meat after cooking was generally considered to be quite satisfactory.

Table 1 - Monthly Palatability Scores for Whole Lobsters and Picked Lobster Meat Stored at 0° F.

Experimental condition of sample	Lot number	Palatability Score[1/] Storage Period (Months) 1	2	3	4	5	6
Frozen whole lobsters							
Uncooked	1	91	87	91	93	89	93
Cooked	2	74	88	84	72	82	83
Cooked (frozen in ice block)	3	-	-	88	-	79	83
Fresh control	-	79	81	67	81	96	88
Frozen lobster meat							
Dry pack	4	79	84	81	78	68	73
Brine pack	5	80	89	86	86	83	82
Fresh control	-	83	86	91	89	92	88

1/The palatability score was calculated as follows: The meat was scored on the basis of 1 to 10 points each for appearance, flavor and texture. The flavor score was doubled in order to give additional weight to this factor. The mean as a percent of these scores resulted in the value reported in the table. A score of 85 or over is considered satisfactory.

The frozen cooked lobsters received variable scores from month to month and in general were not satisfactory. The meat was often spongy and watery, and was not at all like that of fresh-cooked lobsters. The meat of the cooked lobsters frozen in blocks of ice showed no improvement over that of the cooked ones held in the usual manner.

Neither of the lots of frozen picked meat stored too well. The meat that was frozen without added liquid (dry pack) failed to receive an acceptable score during any month of the storage period. The flavor of the meat was objectionable and the texture was described as watery and spongy. The meat frozen with added liquid (brine pack) received somewhat higher scores but in general it could hardly be considered satisfactory either. It is doubtful if this pack has very much in its favor over the dry-packed product.

In all lots, the claw meat was sometimes found to have somewhat of an off flavor, even though the body meat was all right.

Considerable variation was found in the scores for the fresh controls. This may have been due to the manner in which the live lobsters were handled.

While the frozen uncooked whole lobsters received the highest palatability scores, there is one other factor that must be considered. After cooking the whole

lobster, the meat was found to stick very tightly to the shell and was difficult to remove without breaking it into small pieces. This may or may not be objectionable, depending upon the attitude of the consumer, since the meat is very often cut into smaller pieces before being used.

Note: A UNITED STATES PATENT HAS RECENTLY BEEN GRANTED WHICH DEALS WITH THE FREEZING OF LOBSTERS. IT IS CLAIMED THAT THE DIFFICULTIES ENCOUNTERED IN REMOVING THE MEAT FROM THE SHELL OF LOBSTERS FROZEN ALIVE OR RAW AND THEN COOKED MAY BE OVERCOME BY A BRIEF HEAT TREATMENT PRIOR TO FREEZING. THIS TREATMENT CONSISTS OF AN IMMERSION OF THE LOBSTER IN BOILING WATER FOR AN INTERVAL OF NOT LESS THAN 15 SECONDS NOR MORE THAN 5 MINUTES, DEPENDING ON THE SHELL THICKNESS. THE HEATING PERIOD SHOULD BE ONLY OF SUFFICIENT DURATION TO PRODUCE A COOKING OF THE SURFACE OF THE MEAT NEXT TO THE SHELL BUT NOT TO COOK THE MEAT BELOW THE SURFACE. A HEATING PERIOD OF ABOUT 1-1/2 MINUTES IS SAID TO BE SUFFICIENT FOR A ONE POUND LOBSTER. AFTER HEATING, THE LOBSTER IS COOLED AND QUICK FROZEN (U. S. PATENT NO. 2,501,655 "FROZEN LOBSTER METHOD," PTD. MARCH 28, 1950).

--S. R. Pottinger, Chemist,
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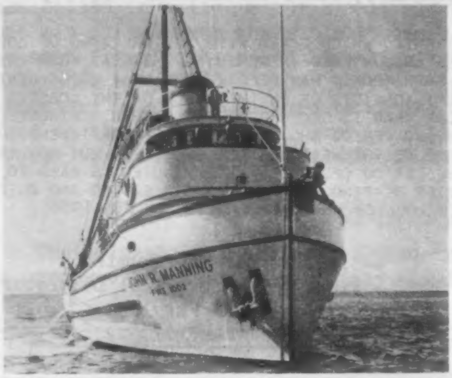
TUNA TROLLING IN THE LINE ISLANDS IN THE LATE SPRING OF 1950

In the normal course of scouting for suitable purse-seine schools of tuna, the John R. Manning, one of the Service's Pacific Oceanic Fishery Investigations research vessels, trolled 5 to 7 lines as a secondary and incidental procedure during a two-month survey in the Line Islands during the late spring of 1950. The ind'cations of possible good production of tunas with surface trolling and the experience gained in surface trolling methods for Line Island tunas are discussed in Fishery Leaflet 351, Tuna Trolling in the Line Islands in the Late Spring of 1950.



This 32-page leaflet points out that good surface trolling was not unexpected in the Line Islands (Kingman Reef, Palmyra, Washington, Fanning, and Christmas), but the John R. Manning was unprepared both in quantity and strength of trolling gear for the size of fish encountered and for the number of hours devoted to trolling. It is the consensus of observers aboard the research vessel that a standard trolling boat, familiar with the area and properly rigged, would have been able to double, at the very least, the catch of the John R. Manning.

The vessel trolled a total of 285½ hours, catching 882 yellowfin (Neothunnus macropterus) weighing 29,319 pounds. Thus, the average weight for all yellowfin was 33.9 pounds. A total of 178 wahoo or ono (Acanthocybium solandri) were taken, estimated at 5,888 pounds. In addition, a small number of skipjack (Katsuwonus pelamis) and miscellaneous fish were trolled, including rainbow runners (Elagatis bipinnulatus) and barracuda (Sphyraenidae).

Included in the leaflet is a description of the gear and rigging used, and trolling operations and results as well as tables giving detailed catch data.

Copies of Fishery Leaflet 351 are available free upon request from the Division of Information, U. S. Fish and Wildlife Service, Washington 25, D. C.

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